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Research Article

Microhabitat use by endangered Iberian cyprinids nase *Iberochondrostoma almacai* and chub *Squalius aradensis*

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Abstract. Over the last decade there has been a major rise in the number of attempts at fish conservation and management as a response to the widespread degradation of aquatic ecosystems. The assessments involved are rarely planned and executed with inputs from the species' life history, particularly their microhabitat use over space and time. The seasonal and size-related microhabitat use of two critically endangered cyprinids – the Iberian nase *Iberochondrostoma almacai* and Iberian chub *Squalius aradensis* – was examined at seven sites across four small catchments in southwest Portugal. Both species displayed non-random microhabitat use. In autumn, nase preferred more sheltered (> 50 % cover) habitats with small substrata (organic cover and silt) than in summer, while chub were found to occupy significantly faster-flowing habitats areas (> 10 cm/s) with coarser substrata (> 50 mm particle size) in the spring than in the rest of the year. Size-related analyses indicated that young-of-year (yoy) nase used coarser substrata (> 5 mm particle size) and more exposed habitats (< 50 % cover) than adult nase. Adult chub, on the other hand, occupied coarser substrata (> 50 mm particle size) and faster-flowing areas (> 10 cm/s) than yoy and juveniles. Based on these findings, it was possible to assign the species to ecological guilds and to classify them as limnophilic (nase) and eurytopic and lithophilic (chub). Both species generally occupied distinct microhabitats, although resource-use overlap was significant in summer. During this season, overlap was found between yoy nase and chub, juvenile nase and juvenile/adult chub, and between adults of both species. The present study identified key factors in the species' habitat requirements and helped develop management recommendations for river restoration that may have a wider application, particularly for other Mediterranean-type rivers.

Key words. Microhabitat use; Iberian nase; Iberian chub; principal component analysis; overlap; fisheries management.

Introduction

Rivers are among the ecosystems that have been most damaged by human activities all over the world (Saunders et al., 2002). Mediterranean rivers are especially susceptible to degradation due to high human settlement and intensive agricultural production, which are in turn the result of mild winters and an

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abundance of sunshine (Gasith and Resh, 1999). There is consequently a high demand for freshwater, in particular for irrigation. Concern about ecological, social and economic losses caused by stream degradation has recently stimulated major conservation and managements efforts (Dudgeon et al., 2006), which are particularly difficult when water quantity is the issue. However, if their goals are to be achieved, such studies should be based on specified guiding images of dynamic, undisturbed rivers (Matthews, 1998). Although the historically high level of intervention they have suffered mean that pristine conditions rarely occur in Mediterranean rivers, some still enjoy a high conservation value due to the absence of major human impacts. As a result, their value when it comes to guiding future conservation studies is of great importance. This brings the need to understand species' life history and their microhabitat preferences in terms of space and time (Lake et al., 2007).

Iberian nase *Iberochondrostoma almacai* Coelho, Mesquita & Collares-Pereira (hereafter nase) and Iberian chub *Squalius aradensis* Coelho, Bogutskaya, Rodrigues & Collares-Pereira (hereafter chub) are both critically endangered (CR) species. They are found exclusively in small basins in southwest Portugal and their ecology at the microhabitat scale is yet largely unknown (Cabral et al., 2006). Quantifying their microhabitat preferences and grouping the species into ecological guilds are essential requisites as inputs for proper fish conservation and management. This information could also prove extremely useful elsewhere, namely in other typical Mediterranean-type rivers, where knowledge of the specific habitat requirements of other nases (*Iberochondrostoma* spp.) and chubs (*Squalius* spp.) is scarce and poor.

The present study aimed to explore the microhabitat use of nase and chub, accounting for season and size-class in order to understand both species' habitat requirements and draw up recommendations for future management studies, such as river restoration. Specifically, we asked the following questions: (1) do species select specific microhabitats (i.e., do species display nonrandom microhabitat use)?; (2) do species display seasonal or ontogenetic differences in microhabitat use?; and (3) do species display microhabitat use overlap?

Materials and methods

Study area

The study area is situated in southwest Portugal (Fig. 1). Seven sites were selected in the Mira (1576 km^2, 1 site), Seixe (258 km^2, 1 site), Aljezur (183 km^2, 1 site) and Arade (987 km^2, 4 sites) catchments. The first three discharge into the sea along the southwest coast, and are dominated by slates and graywackes along the middle reaches, with volcanic igneous deposits occurring in the headwaters. The Odelouca River is the largest tributary of the Arade basin, which is situated in the Algarve region of southwest Portugal. It is a medium-sized low-gradient river dominated by schistose rocks, with alluvial deposits in the lower reaches. Three sites were located in the upper-middle stretch of the main river course, upstream from a partially constructed water-supply dam, which is scheduled to begin operating by 2010. The other site was located on the Carvalho River (28 km^2), a small tributary of the Odelouca. The climate is Mediterranean pluviseasonal oceanic, with rivers that experience considerable flow changes in a typical Mediterranean cycle (Gasith and Resh, 1999). All the sites within the study area are forested, with well-developed riparian galleries dominated by *Alnus glutinosa* L., *Salix salviifolia* Brot. ssp. *australis* Franco, and *Fraxinus angustifolia* Vahl, and are free of major human impacts such as urban pollution, impoundment, and angling. Nase and chub were the dominant species throughout the study sites; they are allopatric in catchments that discharge along the southwest coast. Chub are present in the Seixe and Aljezur catchments, while nase is restricted to the Mira catchment. Both species occur sympatrically in the Arade catchment. Other species occurred sparsely and in small numbers, and included the Iberian barbel *Barbus sclateri* Gunther, the European eel *Anguilla anguilla* L., and the Iberian loach *Cobitis paludica* de Buen. Potential predators include the otter *Lutra lutra* L. (Beja, 1996), and birds – particularly the kingfisher *Alcedo attis* L., the grey heron *Ardea cinerea* L. and the little egret *Egretta garzetta* L.

Fish sampling. The study took place during the flowing season, which typically runs from mid-October to early July, when there is full connectivity between habitats (riffles, runs, pools) and fishes are therefore not restricted to isolated pools. Samplings were conducted by electrofishing at all sites in mid-spring (26–29 April 2006, hereafter spring), early summer (19–22 June 2006, hereafter summer) and late autumn (12–15 December 2006, hereafter autumn), for a total of 21 surveys. A 250-m long study section was chosen on each river, on the basis of accessibility and its representativity of major habitat types (riffles, runs and pools). Fish sampling was then undertaken during daylight hours using pulsed DC electrofishing (SAREL model WFC7-HV, Electracatch International, Wolverhampton, UK), with low voltage (250V) and a 30 cm diameter anode to reduce the

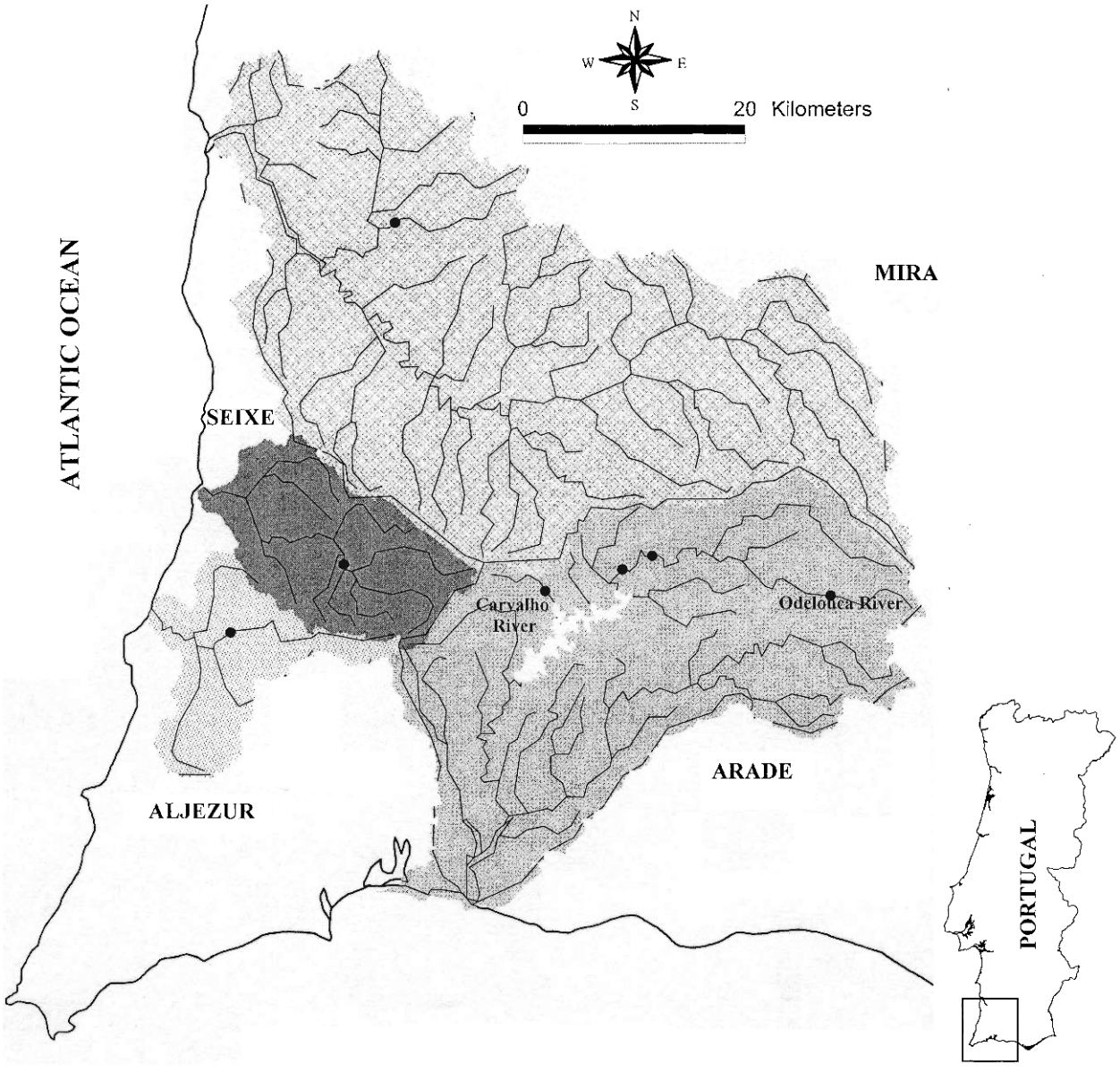

Figure 1. Map of the study area (southwest Portugal), showing the limits of the main catchments (Mira, Seixe, Aljezur and Arade) and the location of the 7 sites that were seasonally sampled in mid-spring, early summer and late autumn 2006 (black dots). A partially constructed water supply dam on the Odelouca River, including the future reservoir pool level, is also shown.

effect of positive galvanotaxis. This equipment made it possible to catch a large range of fish sizes, but did not catch larvae, for which specific protocols are needed (Copp, 1989). Fish sampling was carried out by two people – one operating the shocker, the other using a dip net to catch stunned fish. The sampling crew moved upstream in a zigzag pattern to ensure full coverage of all habitats. A modified point electrofishing procedure (Bain et al., 1985; Copp, 1989) was adopted to avoid pushing the anode forward in the water and causing displacements of individuals from their original position. Sampling points were approached discreetly, and the activated anode was swiftly immersed in the water for 5 s at equidistant locations (every 0.5 m) along cross-sectional transects spaced 1 m apart along the stream section in question. Upon the first sighting of a fish or a shoal of fishes, a numbered location marker was anchored to the stream bed for subsequent microhabitat use measurements. Fish were immediately collected by means of a separate dip net held by another operator, and quickly measured for total length (TL). Captured fish were then temporarily kept in buckets with portable ELITE aerators to avoid continuous shocking and repeated counting. All fish were returned alive to the river at the end of the sampling. To account for ontogenetic

differences in microhabitat use, and as all samplings took place just after both species' breeding seasons (March to May), fish were stratified into three size-classes based on reported differences in length and age structure (Magalhães et al., 2002): <5, 5–7 and >7 cm for nase and <4, 4–6, >6 cm for chub. These roughly correspond to the fish life-stages young-of-year (yoy, 0+), juveniles (1+), and adults (>1+), respectively.

Microhabitat measurements. After fish sampling, four microhabitat variables were measured for each fish position: depth of water column, mean water velocity, substratum composition (dominant substratum size), and percentage cover. Depth was measured with a meter rule to the nearest centimetre. Water velocities were measured with a water flow probe (model FP101, Global Water Instrumentation, Inc., USA). For depths less than 0.8 m, mean water velocity was measured at 60 % of the distance from the surface to the substratum; otherwise, water velocity was considered to be the mean of measurements taken at 20 % and 80 % of total depth (Bovee and Milhous, 1978). Substratum composition and percentage cover were determined visually in 0.8 x 0.8 m quadrats directly below the fish. Substratum was measured according to a modified Wentworth scale (Bovee, 1986) [(1) organic cover; (2) silt (1–2 mm); (3) sand (2–5 mm); (4) gravel (5–25 mm); (5) pebble (25–50 mm); (6) cobble (50–150 mm); (7) boulder (>150 mm) and (8) bedrock]. Cover was defined as any structure inside the water (logs, tree roots, dead branches, submerged and emergent macrophytes <50 cm above water surface) that could afford protection to fish and could not be included in the substratum types. The percentage of cover at each point (in 10 % increments, from 0 to 100 %) was estimated subjectively. Microhabitat availability measurements were made using the same variables by quantifying randomly selected points along 15–25 equidistant transects perpendicular to the flow at each sampling site.

Data analysis

Principal component analysis (PCA) employing resource use and availability data was conducted to test for the presence of nonrandom microhabitat use by nase and chub, and to examine seasonal variation in microhabitat use by both species, partitioned by size-classes. This technique is useful for identifying microhabitat variables that best delimit predetermined species (Copp and Vilizzi, 2004). Varimax rotation was used to facilitate interpretations, and only components >1 were retained for analysis (Kaiser criterion). Loadings $> |0.60|$ were used for interpretation of environmental gradients. Factorial ANOVA was then performed on canonical scores to test the null hypotheses that: (1) season had no effect on microhabitat use; (2) size-class had no effect on microhabitat use; and (3) effects of season and size-class did not interact. Tukey post-hoc tests were used for pairwise comparisons when an overall significant effect was detected. This technique has been used elsewhere to look for significant differences in mean canonical scores between use-availability data and among different size-classes (Baltz et al., 1991; Mäki-Petäys et al., 1997; Santos et al., 2004). Prior to PCA, all data were either log10 (x + 1) (linear measurements) or arcsin[sqrt(x)] (percentages) transformed to improve normality.

Spatial overlap in terms of seasonal microhabitat use by the different species size-classes was assessed using Schoener's Index (C) (Schoener, 1970) at sites where the species occurred sympatrically (Arade catchment):

$$C = 1 - \tfrac{1}{2} \sum_{n=1}^{i} |Pxi - Pyi|$$

where Px and Py are the proportions of species size-classes x and y using habitat i. C ranges from 0 (no habitat overlap) to 1 (full habitat overlap). Overlap is generally considered significant when C exceeds or equals 0.60 (Zaret and Rand, 1971; Sánchez-Gonzáles et al., 2001). Microhabitat types were defined as all possible combinations of depth (two categories, i.e. shallow, $\leq$ 40 cm, and deep, >40 cm), velocity (no current, $\leq$ 5 cm/s, and current presence, >5 cm/s), dominant substratum (depositional, $\leq$ 5 mm particle size, and erosional, >5 mm particle size), and cover (exposed $\leq$ 30 %, and sheltered, >30 %). Cut-off values for microhabitat variables were defined a priori based on previous studies (Godinho et al., 1997; Pires et al., 2004). This gave a total of 16 habitat types.

Results

Microhabitat availability and use

A total of 367 nases [mean total length (TL) 6.2 ± 2.6 cm SD] and 2015 chubs (mean TL 5.1 ± 3.0 cm) were captured during the study period (Table 1). Together the species constituted 96.8 % of total captures. Other species occasionally captured included eel, loach, and barbel. At the study sites, available water depth and velocity for the two species ranged from 5 to 160 cm and from 0 to 155 cm/s, respectively. Substratum was mainly composed of pebbles and cobbles, with some silting taking place in pool habitats. The availability of cover for fish was generally low (<20 %), and included logs, roots, and

Table 1. Seasonal microhabitat availabe to, and use by nase and chub (partitioned by life-history stages) in catchments of southwest Portugal.

Season		Depth (cm)	Water vel. (cm/s)	Dominant subs. (class)	Cover (%)	N
Mid spring	Nase					
	Availability	32.1 (1.4)	5.2 (0.8)	4 (1–8)	10 (0–100)	257
	Use					
	yoy	33.1 (2.7)	0.3 (0.2)	4 (2–6)	60 (0–80)	7
	juvenile	44.5 (2.0)	0.4 (0.3)	3 (1–8)	80 (40–100)	33
	adult	48.7 (2.2)	0.1 (0.1)	3 (1–7)	70 (10–100)	54
	Chub					
	Availability	29.3 (1.1)	11.5 (1.0)	5 (1–8)	10 (0–100)	403
	Use					
	yoy	30.5 (2.5)	5.3 (1.5)	6 (2–8)	10 (0–80)	12
	juvenile	41.6 (1.6)	10.2 (1.6)	6 (3–7)	50 (0–90)	75
	adult	42.3 (1.0)	14.4 (1.1)	6 (1–8)	40 (0–100)	295
Early summer	Nase					
	Availability	30.6 (1.3)	4.3 (0.6)	5 (1–8)	10 (0–100)	422
	Use					
	yoy	39.7 (2.2)	0.5 (0.3)	5 (1–8)	40 (0–100)	68
	juvenile	40.9 (2.5)	0.5 (0.4)	5 (1–8)	70 (10–100)	42
	adult	55.9 (3.1)	0.7 (0.5)	2 (1–8)	70 (20–100)	60
	Chub					
	Availability	29.0 (1.1)	6.4 (0.6)	6 (1–8)	10 (0–90)	497
	Use					
	yoy	18.7 (0.6)	5.0 (0.3)	5 (2–8)	10 (0–90)	529
	juvenile	33.0 (1.4)	6.1 (1.0)	5 (2–8)	10 (0–90)	102
	adult	43.8 (1.0)	10.8 (0.9)	6 (1–8)	50 (0–100)	349
Late autumn	Nase					
	Availability	47.5 (1.9)	28.0 (1.9)	5 (1–8)	10 (0–100)	350
	Use					
	yoy	51.1 (3.6)	3.9 (1.3)	1 (1–8)	50 (0–100)	34
	juvenile	55.6 (3.2)	7.7 (1.6)	1 (1–8)	70 (10–100)	38
	adult	55.8 (3.4)	9.1 (2.5)	1 (1–6)	70 (20–100)	31
	Chub					
	Availability	42.8 (1.5)	37.5 (1.8)	5 (1–8)	10 (0–100)	426
	Use					
	yoy	35.5 (0.9)	7.4 (0.6)	3 (1–8)	20 (0–90)	285
	juvenile	43.0 (1.3)	9.2 (0.9)	3 (1–8)	30 (0–100)	207
	adult	44.8 (1.7)	10.2 (1.1)	2 (1–8)	60 (10–100)	161

Note: Mean values are given for depth and water velocity followed by standard error (in parentheses), while median values (with range given in parentheses) are given for dominant substratum size (1, organic detritus; 2, silt; 3, sand; 4, gravel; 5, pebble; 6, cobble; 7, boulder and 8, bedrock) and cover (in 10 % increments).

overhanging vegetation along the stream banks. Overall, on average nase used deeper microhabitats (mean = 48.2 ± 19.8 cm SD) than chub (35.1 ± 19.3 cm) ($t = 11.9$, $df = 2380$, $P < 0.01$). Significant differences were also found in the mean velocities used by the different species ($t = -9.4$, $df = 2380$, $P < 0.01$). Chub used faster-flowing microhabitats (9.1 ± 13.5 cm/s) than nase (2.3 ± 6.8 cm/s). The species also differed in their substratum use (Mann-Whitney *U*-test, $P < 0.01$), as chub were found to use coarser substrata (median = class 5) than nase (median = class 2). Finally, nase favoured stream areas with a higher percentage of cover (median = 70 %) than chub (median = 30 %) (Mann-Whitney *U*-test, $P < 0.01$).

Nonrandom microhabitat use by nase and chub

PCA on microhabitat availability and use by nase extracted two principal components (PCs) with eigenvalues >1, which explained 68.5 % of the variance in the data (Fig. 2). Substratum (0.89) was positively loaded and cover (–0.75) was negatively loaded on the first principal component (PC1). Nase was found to select specific microhabitats, as this species displayed nonrandom microhabitat use on this component (Mann-Whitney *U*-test on PCA scores, $P < 0.001$). As such, nase were over-represented in sheltered areas presenting depositional substrata. Depth (0.90) was the only variable that loaded high on PC2. A nonrandom pattern was also found on this component, as nase were significantly over-represented in deep areas (Mann-Whitney *U*-test on PCA scores, $P < 0.001$).

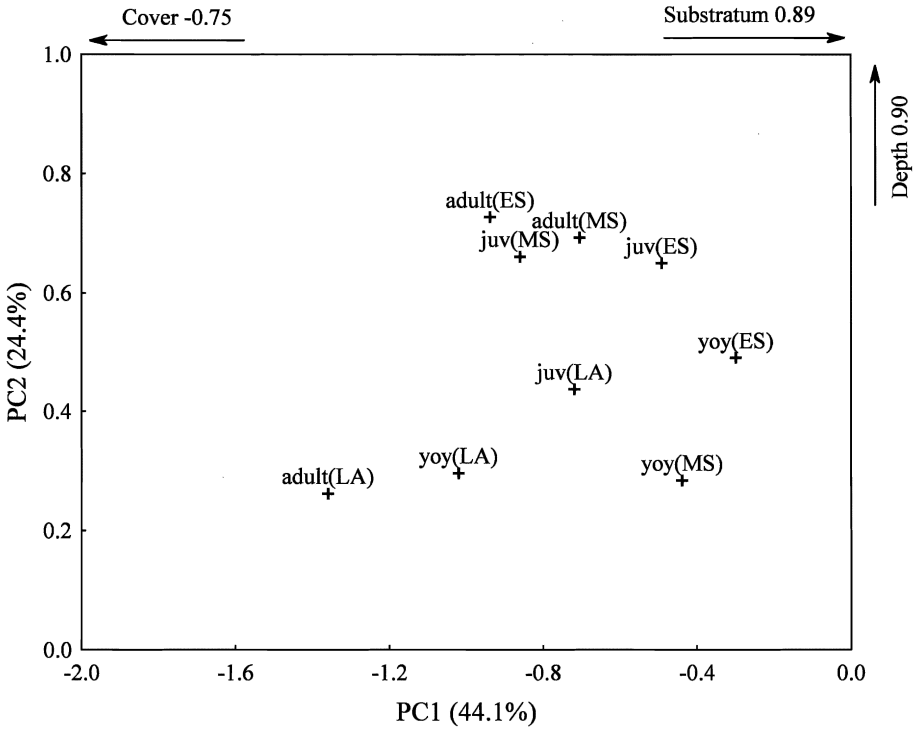

Figure 2. Principal component analysis (PCA) of seasonal and size-related changes in microhabitat use by nase. Mean PCA scores are presented for species size-classes (yoy: young-of-year; juv: juveniles; adults), in mid-spring (MS), early summer (ES) and late autumn (LA). Variables with loadings $> |0.60|$ and percentages of variance explained by each component are also given.

PCA on microhabitat availability and use by chub yielded two components with eigenvalues >1, which explained 67.5 % of the variance in the data (Fig. 3). PC1 had high loadings on depth (0.87) and cover (0.79). Nonrandom microhabitat use was reported for chub in relation to this component, as this species was found to be over-represented in deep, sheltered positions (Mann-Whitney *U*-test on PCA scores, $P < 0.001$). PC2 loaded high on substratum (0.81) and water velocity (0.74). Again, nonrandom microhabitat use was found on this component, as chub were significantly over-represented in low-velocity habitats with small substrata (Mann-Whitney *U*-test on PCA scores, $P < 0.001$).

Seasonal and ontogenetic variation in microhabitat use

Factorial ANOVA on canonical scores of PC1 revealed significant seasonality ($P < 0.001$) – nase shifted to more sheltered areas (>50 % cover) with small substrata (organic cover and silt) from summer to autumn – and size-class ($P < 0.01$) – yoy used coarser substrata (>5 mm particle sizes) and more exposed habitats (< 50 % cover) than adults – effects (Fig. 2, Table 2). However, there was also a significant season-by-size-class interaction effect ($P < 0.05$), thereby indicating that the season affected nases' microhabitat use differently depending on size-class. We found evidence of adults using more covered areas with smaller substrata during autumn, compared to yoy and juveniles in summer. A significant seasonal effect on PC2 was detected, as there was a shift of nase to shallower waters from summer to autumn (ANOVA on PCA scores, $P < 0.01$).

A significant seasonal effect on PC1 was detected, as chub used deeper and more covered areas in spring than in the other seasons (factorial ANOVA on canonical scores, $P < 0.001$) (Fig. 3, Table 2). The effect of size-class alone was also significant, as larger size-classes tended to use deeper and more sheltered positions than smaller ones ($P < 0.001$). Average scores for yoy and juveniles during the summer and autumn were consistently lower than for adults, hence the significant interaction effect between season and size-class ($P < 0.001$). A significant seasonal effect on PC2 was reported, as chub shifted to faster-flowing habitats with coarser substrata in spring (factorial ANOVA on canonical scores, $P < 0.001$). A significant size-class effect was also detected ($P < 0.01$), as larger size-classes used more such resources than smaller ones. There was evidence of interaction between season and size-class, as individuals from the two largest classes shifted to slow-flow and small substratum positions from summer to autumn ($P < 0.01$).

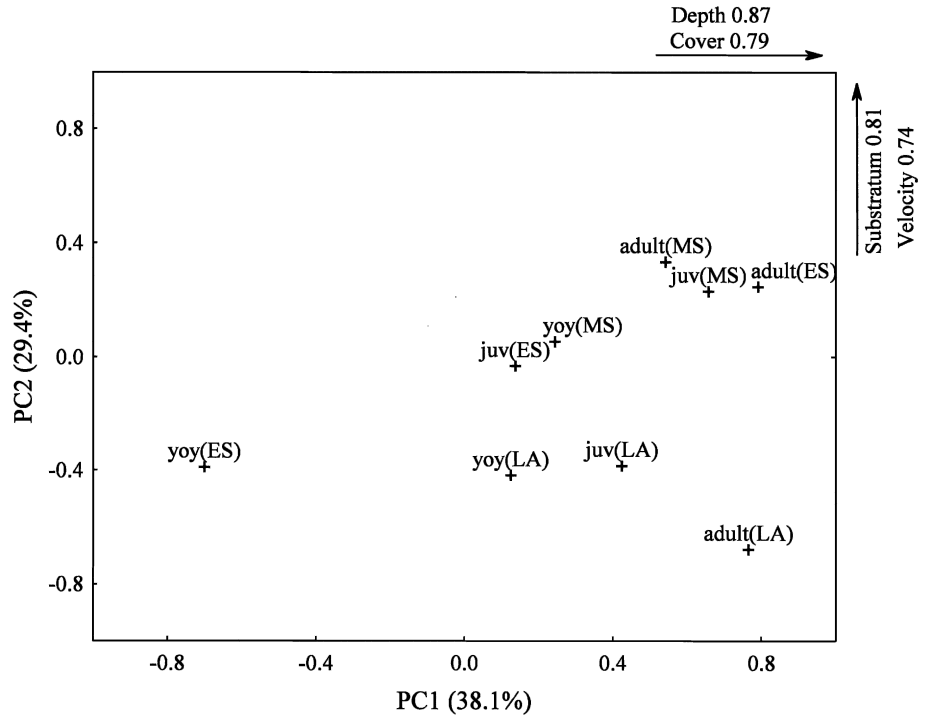

Figure 3. Principal component analysis (PCA) of seasonal and size-related changes in microhabitat use by chub. Mean PCA scores are presented for species size-classes (yoy: young-of-year; juv: juveniles; adults), in mid-spring (MS), early summer (ES) and late autumn (LA). Variables with loadings $> |0.60|$ and percentages of variance explained by each component are also given.

Table 2. Factorial ANOVAs for the effects of season and fish size-class on the microhabitat use of nase and chub in relation to extracted principal components (PC).

Species	PC	Source of variation	F	df	P
Nase	1	Season	8.35	2	< 0.001
		Size-class	5.56	2	< 0.01
		Season x size-class	2.92	4	< 0.05
	2	Season	5.87	2	< 0.01
		Size-class	2.04	2	ns
		Season x size-class	0.73	4	ns
Chub	1	Season	35.28	2	< 0.001
		Size-class	52.78	2	< 0.001
		Season x size-class	22.74	4	< 0.001
	2	Season	40.93	2	< 0.001
		Size-class	22.21	2	< 0.01
		Season x size-class	14.54	4	< 0.01

Microhabitat use overlap

Schoener's indices of resource-use overlap between species size-classes varied with season (Table 3). Overlap was low in spring (overall mean $\pm$ SE $=$ 0.38 ± 0.04, n $=9$) and autumn (overall mean $\pm$ SE $=$ 0.46 ± 0.05, n $=9$), and higher in summer (overall mean $\pm$ SE $=0.61 \pm 0.04$, n $=9$, Mann-Whitney *U*-test: spring, $P < 0.01$; autumn, $P < 0.05$), when almost all pairs of species size-classes revealed significant values (i.e. Shoener's index ≥ 0.60) – particularly yoy nase and chub, juvenile nase and juvenile/adult chub, and adults of both species. The overlap between adult nase and juvenile chub during this season was not significant, but nonetheless high (Schoener's index >0.50).

Discussion

Our results demonstrate that both species generally occupied statistically distinguishable microhabitats, although habitat overlap was found to occur, partic-

Table 3. Schoener's indices of microhabitat use overlap between species-size classes during mid-spring, early summer and late autumn.

Species pair	Season		
	Mid spring	Early summer	Late autumn
yoy Nase / yoy Chub	0.53	0.67*	0.50
yoy Nase / juv Chub	0.36	0.66*	0.58
yoy Nase / adult Chub	0.29	0.61*	0.52
juv Nase / yoy Chub	0.33	0.47	0.35
juv Nase / juv Chub	0.39	0.65*	0.52
juv Nase / adult Chub	0.45	0.83*	0.65*
adult Nase / yoy Chub	0.17	0.38	0.14
adult Nase / juv Chub	0.42	0.53	0.34
adult Nase / adult Chub	0.45	0.66*	0.56

* values above or equal to 0.60 are considered significant

ularly in summer. Nase nonrandomly occupied deep (all size classes) and sheltered microhabitats with small substrata, particularly pool habitats, suggesting that this species has a limnophilic behaviour. Our results differed from those of Magalhães et al. (2002), who found nase to be a habitat generalist. However, the latter study was only focused on the dry season, when the rivers lack continuous surface water and are composed of a series of fragmented sections. Habitat associations that are addressed under such conditions should not be considered to reflect the optimal habitat (Gorman and Karr, 1978), since they represent an externally imposed displacement towards sub-optimal conditions. If they are to be successful, management actions and restoration schemes in Mediterranean-type rivers should therefore look for information on species' habitat use in both the flowing and drying seasons.

Nase used more covered and deeper areas than chub, and also displayed a preference for depositional substrata. It is possible that such patterns may be linked to trophic adaptations (García-Berthou, 1999), as species diet has been shown to influence microhabitat use (Garner, 1996; Nunn et al., 2007). It is clear that future studies should include the influence of food resources in an attempt to better understand the patterns of microhabitat use by both these cyprinids. Overall, chub were found to display non-random microhabitat use, although careful attention should be paid when analysing differences between seasons, size-classes, and the combination of both these factors, as a wider range of conditions is likely to be used depending on the season and ontogeny. This fact indicates that chub may be a eurytopic (generalist) species that inhabits a broad range of habitats. This is also supported by the observed shift towards higher velocity areas with coarser substrata in spring, which is probably related to the reproductive period (March to May). Similar findings have been reported for other Iberian chub species (Pires et al., 2000; Maia et al., 2006), thus suggesting that this species may be a lithophilic spawner that seasonally undergoes small-range spawning migrations.

Cover and depth were the most relevant variables in microhabitat use by nase, whereas water velocity and substratum were more important for chub, as seasonal shifts in these variables were not attributable to concurrent availability differences. Santos et al. (2004) found that water velocity and depth were the principal variables in determining the microhabitat use of *Squalius carolitertii* – a "sister" species of *Squalius aradensis*. On the other hand, Grossman and de Sostoa (1994a, b) found that substratum-related variables played a major role in microhabitat use of fishes in another Iberian river. Above all, it appears that a number of different variables interacted strongly and dynamically in the use of microhabitats by nase and chub.

Significant size-related differences in the species' microhabitat use were found to occur. Larger chub size-classes were detected in deeper and more covered areas than those used by their smaller counterparts. It is possible that predation may have influenced such shifts in depth distribution, as has been reported elsewhere (Copp, 1992; Rosenberger and Angermeier, 2003). As otter and birds are size-selective predators that preferentially consume larger fish (Prenda et al., 2002), an increased predation risk in shallower areas would account for the presence of larger size-classes in deeper and sheltered positions. The presence of smaller specimens occupying shallower positions may also be linked to a reduction in predation pressure (Copp, 1992; Copp, 1999). Although the study area does not contain piscivorous fish that would be likely to chase shoals of smaller fish up to shallower areas (Copp, 1992), the observed patterns could reflect an anti-predator response to otter activity, which seems to concentrate in deeper areas (Godinho et al., 1997). However, it should be noted that differences in microhabitat use observed in the present study could also be attributed to other factors, such as time of day, temperature, food availability, presence of competitors, and river discharge regime (Copp and Vilizzi, 2004; Copp et al., 2005).

Unlike chub, larger nase were found to use habitats with smaller substrata. This trend could be caused by the fact that most nase were found in pools, which contained sandy or silty substrata with organic cover provided mainly by the submerged macrophytes *Ranunculus peltatus* Schrank and *Ceratophyllum demersum* L. These features (e.g. vegetation, detritus, etc.) often increase microhabitat diversity and act as cover for fish (Carter et al., 2004). Larger chub

occupied significantly faster-flowing areas than smaller individuals, and were more closely associated with coarser substrata. This pattern of larger specimens occurring in more energetically costly microhabitats has been documented in the literature (Grossman and de Sostoa, 1994a, b; Santos et al., 2004). Cobble substratum may be useful to this species because it provides cover and concealment and also provides better habitat for macroinvertebrates that serve as a food source (Magalhães, 1993).

Our results showed that the species did not seasonally overlap in their resource use except in summer, when evidence of significant microhabitat use overlap (i.e. ≥ 0.60) was found to occur among almost all species size-classes. Similar results have been found for other cyprinid fishes during a period of reduced flow, when the number of deep-sheltered positions decreased (Copp, 1992). Our findings support the hypothesis that by the end of the flowing season, when habitats start to shrink due to receding waters, fish are becoming increasingly confined to a reduced space that may strengthen biotic interactions, thereby resulting in increasing competition for limited resources such as deeper areas, and in a higher vulnerability to predators (Godinho et al., 1997). Depth has long been recognized to be an important factor that overrides fish assemblage structure in Mediterranean rivers (Godinho et al., 2000). The use by larger individuals of deeper areas in summer and the display of a significant overlap index (Shoenner's index ≥ 0.60) may reflect this shortage of surface waters. However, the observed overlap index values are not indicative of trophic separation. In addition to spatial separation, trophic partitioning has also been found to be the one of the main causes of interspecific separation in stream fishes (Ross, 1986). Again, it is necessary that future studies include the influences of feeding strategies, in an attempt to advance our knowledge about the mechanisms that are responsible for the coexistence of the two species.

The assignment of ecological guilds to the target species, as presented in this study, is essential not only to an improved understanding of their habitat requirements, but also to setting recommendations for consideration in river restoration studies elsewhere, particularly in other typical Mediterranean-type rivers, where knowledge of the specific habitat requirements of other cyprinid species (e.g. nases *Iberochondrostoma* spp. and chubs *Squalius* spp.) remains scarce and poor (Doadrio, 2001). Future restoration management should consider increasing the number of sheltered pools, which are a key habitat for nase. This could be achieved by recruiting (allow passive natural input) or placing (active introduction) large wood from nature riparian stands in stream sections. Besides its potential for improving hydromorphology, and its low cost, this method has proven to be a possible measure for river restoration in Central Europe (Kail and Hering, 2005). Provided that sufficient water is ensured by setting adequate minimum flow requirements, using large wood for restoration in Mediterranean rivers remains a promising option. At the same time, the creation of gravel bedforms and artificial riffles in order to benefit lithophilic spawners, such as chub, could be used in future restoration plans, as they have been shown to provide a wider range of depth, velocity and substratum conditions across different flow ranges (Sear and Newson, 2004). Nevertheless, scientists, managers and water users should be aware that species' ability to respond positively to river restoration schemes will also depend firstly on whether water quality in the new scenario is sufficient to support them, and secondly, on whether both species are able to disperse to, and exploit, the improved habitats. The failure of one or both these factors may seriously compromise restoration efforts.

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